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Ecophysiological Studies on *Atriplex farinosa* Forssk. under Different Habitat Conditions

Amal, A. Morsy

Dept. of Botany, Faculty of Science, Ain Shams Univ., Cairo, Egypt.

Abstract: Anatomical features, photosynthetic pigments, protein amino acids, carbohydrates, protein and isozymes were studied in the perennial crynohalophyte *Atriplex farinosa* under the influences of different habitat conditions. samples of *Atriplex farinosa* were collected from three habitats; 5Km north of Mersa Alam**,** 93 Km south of Mersa Alam and Mersa Hemeira at the southern extension of the Red Sea coast in Egypt. The soil texture was differed widely from one locality to another; which could be related to the variation of soil parent material. The stem anatomical features differed clearly between different samples of *A. farinosa*. Protein amino acids showed interspecific and locational variations. The accumulation of total soluble salts was associated with appreciable quantities of certain amino acids such as aspartic acid, glutamic acid, lysine, proline, tyrosine, phenylalanine and amino butyric acid. The photosynthetic pigments, carbohydrates and crude protein attained their higher levels under higher saline conditions (93 Km south of Mersa Alam). The total number of protein bands was nine which were not necessarily detected in all habitats. Five monomorphic ones with approximately molecular weights of 56, 31, 24, 18 and 11 KDa were recorded in all populations which could be considered as a positive marker. The highest similarity matrix of protein profile was (65.5%) between two populations from 93 Km south of Mersa Alam and Mersa Hemeira*,* while the lowest one was (21.8%) between populations from 5Km north of Mersa Alam and 93 Km south of Mersa Alam. Native polyacrylamide gel electrophoresis was used to identify the isozyme variations in α- Amylase, Protease and Peroxidase. The dendrogram based on isozymes revealed two groups of high similarity (66.7%), while the lowest one (11.1%) appeared between *A. farinosa* at 5Km north of Mersa Alam and 93 Km south of Mersa Alam. The position of populations from genetic distance was found to be nearly the same with the geographical position of populations.

Key words: Atriplex farinosa Red Sea coast, ecophysiology

INTRODUCTION

The genus *Atriplex* (Chenopodiaceae) is represented by different species distinguished by various morphological, biological cycles and ecological adaptations (Houerou *et al.,* 1995). *Atriplex* shrubs have adaptations enabling them to tolerate the adverse effects of salts internally, or excrete salt from cells and tissues (McKell, 1994). As a result they have an advantage over other species that lack strategies to deal with salt in the soil, thus excellent competitors in saline environments. In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth (Ashby and Beadle, 1957; Chatterton and McKell, 1969; Matoh *et al*., 1986). Many species of *Atriplex* are valued as livestock forage when herbage availability is low especially in arid environments and salt-affected area (Houerou *et al.,* 1995) because they have high content of crude protein, vitamins (A, C and D) and minerals such as chromium (Shani *et al.,* 1972; Mckell, 1989). Despite *Atriplex* species are considered as a valuable source of forage and are widely distributed in Egypt, few physiological and genetical studies were reported. However it is important to study the effect of different habitat conditions on the physiological and biochemical genetic characterization of *A. farinosa*. Therefore, the main objective of this study is to get a preliminary knowledge of the physiological and biochemical genetic diversity of *A. farinosa* growing under different habitat conditions in Egypt.

Corresponding Author: Amal, A. Morsy, Dept. of Botany, Faculty of Science, Ain Shams Univ., Cairo, Egypt.
E-mail:amorsy70@hotmail.com

MATERIALS AND METHODS

The Study Area:

The Red Sea coastal zone of Egypt extends fromSuez (Lat. 30° N) to Marsa Halaib (Lat. 22° N) near theSudaese – Egyptian borders. Regarding its climatic conditions, the Red Sea is located in a region which is characterized by such an arid climate in which the evaporation from water surface greatly exceeds the little precipitation (Said, 1990). The study areas are shown in Fig (1).

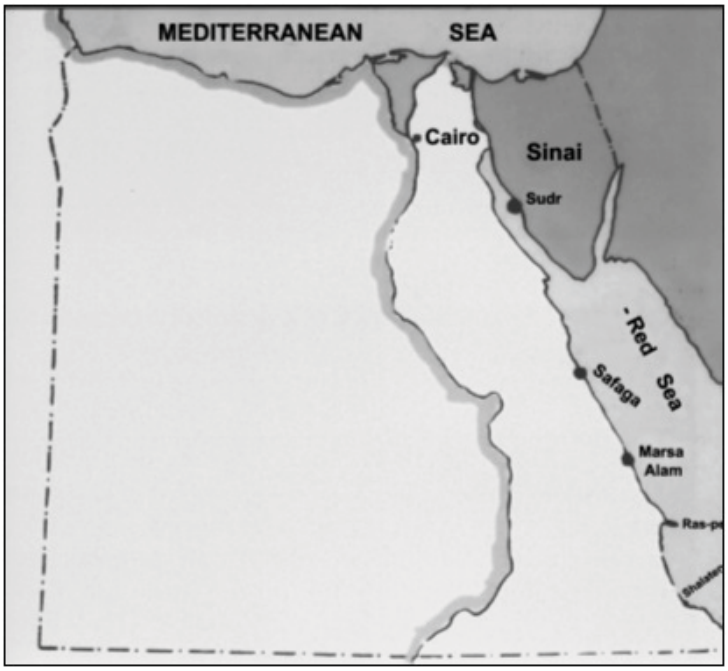


Fig 1: Location map showing the sites form which A. farinosa samples were collected.

Sampling:

Atriplex farinosa Forssk. samples were collected in December 2005 from three different localities in the southern extension of the Red Sea coast in Egypt. (I) 5Km north of Mersa Alam in the site located between Lat 25° 19 28¶ N and Long 34° 52 40¶. (II) 93 Km south of Mersa Alam offshore sandy embankments. It was noticed that *A. farinosa* occupied the coastal strip subject for marine tides. It is located between Lat 24° 21 40¶ N and Long 35° 17 37¶. (III) Mersa Hemeira, 41Km north of Shalatin town within the sand encroached long head located between Lat 23° 28 59¶ N and Long 35° 29 22¶. Floristic composition of the surveyed sites supporting the growth of *Atriplex farinosa* were shown in Table (1). The associated soil profiles were dug wide open to the depths where the greatest amount of plant roots occurred. Accordingly, three soil samples were collected from each site at 0-15, 15-30 and 30-50cm. then air - dried, crushed, sieved through 2 mm sieve and subjected to analysis. The mean values were represented in Tables (2&3).

Table 1: Floristic composition of the surveyed sites supporting the growth of *Atriplex farinosa*

Plant communities	Site I	Site II	Site III
I- *Arthrocnemum machrostachyum*	d		
Suaeda monoica	co-d		
Atriplex farinosa	rr		
II- *Arthrocnemum machrostachyum*		d	
Avicennia marina		ab	
Atriplex farinosa		ab	
III- *Avicennia marina*			d
Atriplex farinosa			

(d) dominant (r) rare (rr) very rare (ab) abundant

Table 2: Granuleometric analysis of the soil samples of *Atriplex farinosa* under studied habitat conditions. Each value is the mean of 3 variables

Habitate	Depth	Coarse sand %	Fine sand %	Silt +Clay %	Texture
I	0-15	10.90	82.10	7.00	Fine sand
	15-30	9.50	84.25	6.25	
	30-50	8.30	90.00	1.70	
II	0-15	15.08	77.80	7.12	Loamy sand
	15-30	18.00	74.20	7.80	
	30-50	16.10	77.00	6.90	
III	0-15	15.50	70.10	14.0	Sandy loam
	15-30	16.42	70.00	13.48	
	30-50	17.00	68.00	15.00	

(I) 5Km north of Mersa Alam (II) 93 Km south of Mersa Alam **(III)** Mersa Hemeira

Table 3: Chemical analysis of the soil samples of *Atriplex farinosa* under studied habitat conditions. Each value is the mean of 3 variables

Habitat	Depth cm	pH	TSS (ppm)	Cationic meq/L				Anionic meq/L			
				Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	CO_3^{--}	SO_4^{-2}
I	0-15	7.36	4456	77.90	1.10	14.5	24.00	66.82	8.54	tr	0.88
	15-30	7.24	4037	83.00	1.50	15.9	29.00	70.0	9.50	tr	0.84
	30-50	7.42	4398	80.00	1.25	13.9	25.33	66.33	7.960	tr	0.78
II	0-15	7.33	10070	887	12.3	35.87	50.54	88.98	77.90	tr	0.80
	15-30	7.40	10500	870	10.3	30.0	57.00	98.0	81.00	tr	0.87
	30-50	7.35	9880	786	11.0	22.73	45.98	90.00	75.50	tr	0.89
III	0-15	7.85	8890	189	5.50	26.01	44.44	124	30.0	tr	0.97
	15-30	7.70	9928	239	6.50	28.1	50.00	128	26.10	tr	1.05
	30-50	7.60	9978	242	6.60	27.22	45.78	121	24.9	tr	1.10

Soil analysis. Particle size distribution was carried out by the dry sieving method of Kilmer and Alexander (1949). Soil reaction was determined in the soil paste (1:5) using a Beckman bench type pH- meter, (Richards, 1954). Total salinity (EC_e) in the soil saturation extract was determined conductimetrically, (Richards, 1954). Cationic and anionic compositions of the soil extract were accomplished according to the methods described by Richards (1954) and Jackson (1962).

Determination of Protein Amino Acids:

Defatted plant powder (0.1g) was extracted first with 80% ethanol, dried and dissolved in 10ml of 6N HCl in a sealing tube, (Awapora ,1948). The mixture was hydrolyzed at 110 °C for 24 hours. Preparation of amino acids for injection in GLC was performed by the method described by Lamin and Gehrke (1966).

Crude protein nitrogen content. Total protein nitrogen was calculated by multiplying the total nitrogen in dry tissues by 6.25. The total nitrogen was determined by using the modified micro-Kjeldahl method of Peach and Tracey (1965).

Photosynthetic pigments. Photosynthetic pigments in fresh leaves were determined quantitatively using the method described by Metzner *et al.,* (1965).

Total carbohydrate. Total carbohydrate content in dry tissues was estimated according to the method described by Chaplin and Kennedy (1994).

Anatomical studies. Fresh materials of *Atriplex farinosa* (the fourth internode, from the stem base were taken and fixed in 70% formaline acetic acid: ethyle alchohol (F.A.A.) .Then washed in 50% ethanol and dehydrated in ethanol-chloroform according to the method outlined by Esau (1960). After complete dehydration the specimens were embedded in pure Paraffin wax (m.p.56 °C) and transverse sections (20-30 µm in thickness) were cut on a rotary microtome. Safrain and fast green method of staining was used, then the sections were mounted in Canada balsam, dried and examined using image processing and system analysis. (Fukui, 1988).

Biochemical Analysis:

Protein Electrophoresis:

SDS- polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins according to the method of Laemmli (1970), and the modification of Studier (1973).

Isozymes electrophoresis. Native – polyacrylamide gel electrophoresis (Native-PAGE) was used to identify the isozymes variation. Accordingly, three isozymes (α- Amylase, Protease and Peroxidase), were extracted from the plants and were separated in 9% polyacrylamide gel electrophoresis according to Stegemann *et al.,* (1985). The gels were stained according to their enzyme system with the appropriate substrate and chemical solution, and then incubated at 37° C in a dark room for complete staining. The staining gels were carried out according to Jonathan and Wendel (1990) and Heldt (1997) for ά- Amylase, Protease and Peroxidase, respectively.

Gel Analysis:

All gels resulted from protein and isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. The densitometry scanning of the bands were performed on three directions. Each band was recognized by its length, width and intensity. Accordingly, relative amount of each band could be quantified and scored.

RESULTS AND DISCUSSION

Plant Description and Distribution:

Atriplex farinosa (Family Chenopodiaceae) is a tall, robust shrub of yellow-white appearance with large, naked panicles, but leaf base cordate with long, obtuse auricles, fruit bracts entire, longer than broad, spathulate, acute (Tackholm, 1974). This chenopod is confined in its distribution to vallies having salty substrate. Its distribution in Egypt is confined to the coastal salt-affected zones along the Red Sea (Tackholm, 1974) and Gebel Elba region (El-Hadidi, 1979) and recorded in salt marshes (Kassas, 1953). In their survey of plant life in the Red Sea littoral zones, Kassas and Zahran (1967) recorded *Atriplex farinosa* in the shore line zone at several localities. Moreover, Ahmed (1996) in his illustrated flora of G. Elba reported that this bush is of common occurrence along the southern littoral zones occupying mostly the shore line where its basal parts may be subject for periodical inundation.

Soil Analysis:

The differences in chemical composition of the plant species in different sites are a function of the edaphic factors (Tadros, 1965). The data of texture and chemical properties of the collected soil samples were recorded.

Soil Texture:

Soil texture widely differed from one site to another (Table 2) where the dominant texture in site (I) is totally fine sand throughout the entire depth of soil profile, while site (II) distinguished by loamy sand texture. The third habitat has soil texture of sandy loam. This variation in soil texture of the selected three localities could be related to the variation of soil parent materials from which these soils were derived.

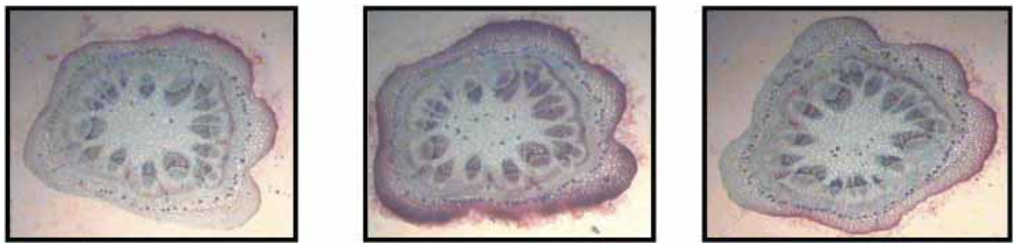
Soil Chemical Properties:

Table (3) shows chemical parameters of the studied soil samples. The obtained data revealed that the pH values were mildly alkaline in reaction in all layers of the studied sites.

The total soluble salt (TTS) showed the highest value (10500 ppm) in the soil of habitat (II).However the lowest value (4037ppm) was recorded in site (I). Na^{+} ion was dominated the chemical composition where represent the highest cation content followed by Mg^{++} and Ca^{++} ions, while K^{+} ion is the least. On the other hand, Cl^{-} ion was the highest anion content followed by HCO_3^{-} and SO_4.

Anatomical Studies:

Table (4) and Figure (2) summarize the differences in stem anatomy .The results clearly show that four main anatomical variants were recorded in the stem of *A. farinosa* of different habitats. The first is that total area of section was between 2.82 - 0.95 mm^2 .The second other width of cuticle ranged from 2 - 3 µ. From table (4), it is clear that epiderms consists of multilayer of radial cells and also show that the highest area was 40.0 % with the highest number of lignified cells in *A. farinosa* at site (II). Whereas the lowest area was 25.0 % with the lowest number of lignified cells in *A. farinosa* at site (I). The results indicate that the different variants in the percentage area of cortex among the studied habitats were shown, where the highest value was 1.93 % at site II and the lowest one was at site (I). Results revealed also small variation in percentage area of vascular cylinder. Plants developed several mechanisms to tolerate salinity and drought such as fleshy cortex in chenopods, presence of multiple epidermis in some cases, presence of great proportion of lignified elements and high ratio of volume to surface. From the former results, it could be possible to differentiate clearly between the different samples of *Atriplex farinosa*. This difference can be the product of genetic responses of their habitats. Also the results have demonstrated that ecological differences can play an important role in the adjustment of this genus to various habitats. It is worth mentioning that the anatomical features of stem of the recognized plants exhibited considerable differences which appeared be the product of the adaptive mechanisms to environment (Batanouny *et al.,* 1991 and Khafagi *et al.*, 1996).



(I) 5Km north of Mersa Alam (II) 93 Km south of Mersa Alam (III) Mersa Hemeira

Fig. 2: Anatomical features of stem of *Atriplex farinosa* under studied habitat conditions

Table 4: Anatomical features of stem of *Atriplex farinosa* under studied habitat conditions

Habitat	Total area mm2	Cuticle thickness µm	Epidermal thickness µm	Cortex		Vascular cylinder		
				Thickness µm	Area mm2	Height mm	Width mm	Xylem length mm
I	0.95	2	25	260	0.71	0.17	0.11	0.09
II	2.82	3	40	345	1.93	0.26	0.15	0.14
III	1.48	2	30	309	0.97	0.21	0.16	0.12

Table 5: Amino acid composition (μg^{-1}100g Fr.wt.) of the studied of *Atriplex farinosa* under different habitat conditions.

Site	Acidic Amino acid		Basic Amino acid			Aromatic Amino			Aliphatic Amino acids								S-Containing	
	Aspartic (µg/ml)	Glutamic (µg/ml)	Lysine	Histadine	Arginin	Proline	Phenyl alanine	Tyrosine	Glycine	Alanine	Valine	Leucine	Iso-leucine	Threonine	Serine	Amino butyric	Cystine	Methionine
I	90.1	84.3	20.7	61.2	__	__	41.3	54.6	59.6	73.6	91.2	18.2	9.4	14.2	23.3	60.7	__	__
II	319.4	88.4	145.8	38.0	__	27.1	203.8	228.4	61.5	41.8	50.1	56.9	34.6	38.6	83.5	251.1	__	__
III	111.4	79.3	61.7	82.3	8.9	11.6	20.2	41.2	70.3	51.6	84.6	90.1	40.3	92.6	80.3	201.3	4.3	3.1

Protein Amino Acids:

Results in Table (5) show the protein amino acid composition of the studied *A.farinosa* in the different habitats. Aspartic acid, glutamic acid, lysine, proline, tyrosine, phenylalanine and amino butyric acid recorded the highest values in *Atriplex farinosa* at site (II).The accumulation of amino acids particularly proline concomitantly with the increase in protein in *Atriplex farinosa* at site (II) could represent an adaptive mechanism for better growth and survival during drought and salinity stresses, Nikolopoulos and Manetas (1991) reported that amino acids could play a role as osmotic regulators and as protective agents for cytoplasmic enzymes.However, Good and Zaplachinski (1994) detected an accumulation of amino acids with a decrease in protein synthesis under water stress which has been described to enhance protease activity, as an adaptive mechanism to stress tolerance. Proline accumulation was regarded by Serrano and Gaxiola (1994) to play a role as nitrogen reserve, to protect protoplasm from dryness and to play an important role in osmoregulation imbalance as a buffer against osmotic imbalance caused by salinity and drought stresses. On the other hand, *A. farinosa* at site (III) was characterized by increased levels of histidine, arginine, glycine, leucine, isoleucine and threonine, as well as the sulfur amino acid cysteine and methionine which were also prominent which might be regarded as a sign of the presence of resistant proteins. In this regard, El-Shourbagy and Abdulla (1975) found an increase in the shoot dry weight and protein content of salt stressed barley seedlings supplied with a mixture of six amino acids; arginine, aspartic, glutamic, cysteine, lysine and tryptophan. However, difference exists in the formation of adaptive protein types in each variety under each habitat. The synthesis of protein types rich in certain amino acids could be the key of survival for the species (El- Shourbagy *et al*, 1980).

Pigment Content:

Chlorophyll a and b and carotenoid contents in the fresh leaves of the *A. farinosa* are presented in Table (6). Results reveal that; in sites (I) and (III) chlorophyll (a and b) ranged in narrow scale while chlorophyll a and b and carotenoids at site (II) showed the largest amount (4.00, 2.12 and 0.56 ug/g ,respectively) .In their studies on the adaptive responses of desert plants under different habitat conditions, Morsy (2002) reported that crynohalophytes attained higher concentrations of photosynthetic pigments under salt stress in their extremely arid habitat and Elabsy (2006) suggested that increase in carotenoids may be considered as one of the adaptive responses which can delay senescence and maintain the survival of stressed plants through protection against oxidative stress.

Table 6: Some chemical constituents (μg^{-1} g F.wt.) of the studied *Atriplex farinosa* under different habitat conditions

Species	Site	Chlorophyll a	Chlorophyll b	Carotenoids	Carbohydrat	Protein
A.farinosa	I	1.90	1.00	0.12	48.5	10.2
	II	4.00	2.12	0.56	77.5	12.6

Total Carbohydrate Contents:

Results indicate that carbohydrates content varied with each habitat and was reached the highest level in *A. farinosa* (77.5 µg/100g) in site (II) (Table 6). In this connection Mohamed and Alain (1995) suggested that accumulation of carbohydrates under salinity stress being due to reduction in their utilization, either as a source of energy or for the formation of new cells and tissues. On the other hand, Cornic and Massacci (1996) and Abo- Kassem *et al* (2002) reported that high salt concentration can result in osmotic adjustment by regulating the accumulation of solutes especially sugars and proteins.

Crude Protein Nitrogen Contents:

Table (6) shows the highest value (12.6 µg/100g dry wt) of total protein N in *A. farinosa* being recorded at site II concomitantly with the highest value of TSS while, at sites I and III plants have the same value (10.2 µg/100g dry wt).In this connection, Ahmed and Girgis (1979) emphasized the importance of nitrogen intermediates as osmotically active ingredients in plant metabolism and showed that desert plants depend, to a large extent, on the accumulation of organic intermediates in building up their osmotic pressure. Nilsen and Orcutt (2000) reported that plants frequently produce a number of unique proteins in their response to environmental stresses

Protein Electrophoresis:

Total protein banding patterns based on SDS-PAGE for *A. farinosa* are illustrated in Table (7) and Figure (3) .The total number of bands was bands which were not necessarily present in all habitats .The bands were detected at approximately molecular weights ranging between 117 and 11 kDa. The resulted profiles comprise three polymorphic bands, which were present in some habitats and absent in the others. Whereas five monomorphic bands with approximately molecular weights of 56, 31, 24, 18 and 11 KDa which may include dehydrins(25.0-60.0 kD) or aquaporins(25.0-30.0 kD) were detected. These proteins may be important in plant adaptation to the desiccation and ionic effects of saline conditions (Yamaguch- Shinozaki *et al*., 1992). Also, it has three bands; one positive and other negative specific bands with Mws 14 KDa., 48 KDa, and 117 KDa, respectively which could be used to distinguish one habitat from the others. The results also revealed that the maximum number of bands was 7 and observed in *A. farinosa* at site (I). These results suggest that production of these stress proteins may represent a common mechanism which enables halophytes to withstand the harmful affect of salinity. Some of the induced proteins might be a group of membrane-bound proteins that effect water transport through membrane and not ions, their abundance in the tonoplasm makes them important for water and ionic balance between the cytoplasm and the vacuole (Youssef *et al*., 2003). The data of SDS-PAGE of total proteins were applied to the computer program SPSS version 10 and a dendrogram for genetic distances was obtained (Fig. 4). The similarity matrix (Table 8) indicates that the highest similarity was (65.5%) between two populations (II) and (III) while the lowest one (21.8%) was between populations from (I) and (III). This combined system could discriminate between the three populations.

Table 7: Banding patterns and molecular weibht (MW) of SDS PAGE Proteins of *Atriplex farinosa* under different habitat conditions. (+) presence of band (-) absence of band

Band No	M.wt. KDa	A. farinosa		
		I	II	III
1	117	+	+	-
3	56	+	+	+
4	48	-	-	+
5	31	+	+	+
6	24	+	+	+
7	18	+	+	+
8	14	+	-	-
9	11	+	+	+
Total		7	6	6

Isozymes Electrophoresis:

In the present study three isozyme systems: (α- Amylase, Peroxidase, and Protease) were studied.

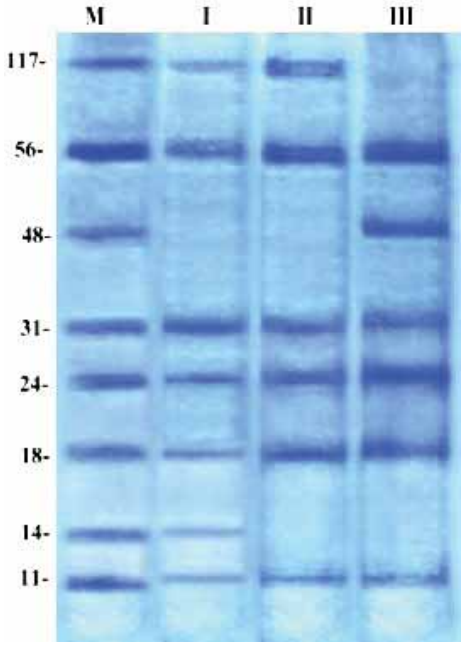


Fig. 3: Proteins profiles of three populations of Atriplex farinosa under different habitat conditions.

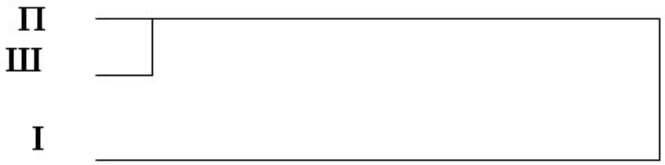


Fig. 4: Dendrogram demonstrates relationship among three populations belonging to *Atriplex farinosa* under different habitat conditions.

Fig. 5: Dendrogram based on Isozyme

α- Amylase:

The pattern of α-Amylase isozyme revealed low polymorphic level, where four bands were expressed among the studied habitats (Table 9). Three bands number 1, 2 and 3 were scored as common bands, with differences in intensity between the three different habitats , while the band number (4) was distinguishable for *A. farinosa* in habitat (I).

Peroxidase:

Table (10) shows the banding patterns of Peroxidase. According to the presence or absence of bands, this iso-enzyme was identified by three bands. Bands number (1) and (3) were scored as common ones, with differences in intensity between the three different habitats, while the band number (2) was distinguishable for habitat (II). Peroxidase has been also reported to be enhanced by water stress and this was positively correlated with water stress tolerance (Hernandez *et al*., 2000 and Turkan *et al.,* 2005). The consistent increase in peroxidase activity in *A. farinosa* at site (II) even during severe salt-stress and its differential regulation, show that it may be more stable or more important for stress tolerance.

Table 8: Matrix of the genetic similarity estimates of protein banding patterns of " Atriplex farinosa"under different habitat condition.

Species	*A. farinose*		
locations	I	II	III
I	,000		
I	,333	,000	
I	,218	,655	,000

Table 9: Electrophoretic patterns of α- Amylase isozyme of *Atriplex farinosa* under different habitat conditions.

Plant	*A. farinosa*		
α- Amylase	I	II	III
1	+	+	+
2	+	+	+
3	+	+	+
4	+	-	-
Total	4	4	3

Table 10: Electrophoretic patterns of peroxidase isozyme of *Atriplex farinos*a under different habitat conditions.

Plant	*Atriplex farinosa*		
Peroxidase	I	II	III
1	+	+	+
2	-	+	-
3	+	+	+
Total	2	3	2

Table 11: Electrophoretic patterns of Protease isozyme of *Atriplex farinosa* under different habitat conditions.

Species	*A. farinosa*		
Protease	I	II	III
1	+	+	+
2	+	+	+
3	+	+	+
Total	3	3	3

Table 12: Matrix of the genetic similarity estimates of isoenzymes of *"Atriplex farinosa"* under different habitat conditions.

Plant	A. farinosa		
Locations	I	II	III
I	,000		
II	,111	,000	
III	,567	,667	,000

Protease:

Results of banding patterns of protease isozyme indicates that there is no polymorphic level, where three bands were expressed among the studied habitats. The three bands were scored as common bands at the three different habitat conditions (Table 11).

In general, results of the above three isozymes indicate that they were identified in *Atriplex farinosa* in the different habitats. Thomas *et al*. (1992) reported that adaptation of metabolic system to high internal NaCl content might be due to the formation of salt tolerant isozymes. Based on the available data of the isozyme patterns,a dendrogram (Fig. 5) was demonstrated. The highest one (66.7%) was between two populations (II) and (III) while the lowest one (11.1%) was between two populations (I) and (II).The position of populations from genetic distance was found to be nearly corresponding to the geographical position of populations.

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